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## Seasonal changes of pH and buffer capacity of aqueous homogenates of *Vaccinium vitis-idaea* and *Vaccinium myrtillus* leaves

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### Abstract

The newly formed leaves of both *Vaccinium* species show in June and July the lowest pH values, 3.4-3.7, which gradually increase up to 4.6 in November. The bi- and triennial leaves of *Vaccinium vitis-idaea* are less acid and show much more limited seasonal decrease of acidity. The buffer capacity against acid of the current year leaves of the two species shows in summer a high level of values (a constant one for *Vaccinium myrtillus*), it decreases in autumn to achieve that of bi- and triennial leaves of *Vaccinium vitis-idaea*. The buffer capacity values are influenced to a limited extent by the increase in air-pollution, much smaller than that noticed for *Pinus silvestris* co-existing with *Vaccinium* in the same ecosystems.

### INTRODUCTION

*Vaccinium vitis-idaea* and *Vaccinium myrtillus* are important components of herbs layer in forests, their appearance being a sign of soil acidification and of the stand impoverishment, and also proving the excessive exposure to light. Most of papers dealing with plants influenced by the industrial pollution are concerned with coniferous trees (e.g. Czuchajowska, Przybylski, 1978; Czuchajowska, Niemtur, 1978), because of their sensibility and liability to severe injuries, while the interest in dwarf shrubs, particularly in *Vaccinium* species strongly influenced by emissions after the thinning out of the forest, is much more limited.

The up to date investigations of this important component of the vegetal cover are mainly concerned with intensity of its growth under

controlled conditions, with phenology (Falińska, 1973; Buszman, Lorek, 1979), the quantity and quality of fruits (Falińska, 1969), anatomy and morphology and also with accumulation of heavy metals (Lorek, 1978; Buszman, Lorek, 1979). Although it has been found that both *Vaccinium* species show different reaction to industrial pollution — as far as the intensity of their annual and radial growth and heavy metals content are concerned (Huttunen, 1975) — no papers dealing with the disturbing influence of industrial emissions on metabolic processes are available. One of the methods of research of these processes is the determination of hydrogen ions concentration and of buffer capacity in leaves collected from polluted zones, because the two factors are to a great extent responsible for many processes substantial for the vital activity of plants.

#### MATERIALS AND METHODS

**Description of the stands.** The three areas from which the leaves were taken to investigation of pH and buffer capacity, lie in close neighbourhood of the zinc-plant at Miasteczko Śląskie (Upper Silesia), namely: the Żyglinek stand in the distance of 0.9 km to north-east from the mentioned pollution center, Brynica I and Brynica II — 2.5 km and 5 km, respectively, to east. They represent stands of different — but always high-degree of pollution of soils and air, see Tab. 1. The fourth area, a control one, containing the previously investigated pines (Czuchajowska, Przybylski, 1978) was located near Pszczyna, 40 km apart of the other stands, to south-east direction. Its soil and air pollution was rather limited, as shown in Tab. 1.

**Materials.** The leaves of *Vaccinium vitis-idaea* and *Vaccinium myrtillus* were being collected as follows. One shoot was cut off of one hundred dwarf shrubs, randomly chosen in every investigation area; in case of *Vaccinium vitis-idaea* every shoot contained the current year, biennial and triennial leaves, while the shoots of *Vaccinium myrtillus* had only the current year leaves. The material was transported in darkened containers to the laboratory, where two or three leaves of every age were torn off from the middle part of the growth. Care was taken to separate the leaves of different age, what was particularly difficult as far as the biennial and triennial ones were concerned. Then, the sets of leaves of every age were divided into two parts. The first one was carefully washed with cold water and destined to pH and buffer capacity measurements, the second part was analysed without previous washing, in order to prove whether — and to what extent — the industrial dusts covering the leaves influenced the determined pH values.

Table 1  
Characteristics of pollution intensity of the investigated *Vaccinium* stands in 1977

Stand	Content of heavy metals in soil upper layer(0—5 cm), ppm				Dust fall, tn/km <sup>2</sup> /month	SO <sub>2</sub> concentration, mg/m <sup>2</sup> /24 h
	Zn	Pb	Cd	Cu		
Żygiełek, Ż	2150	2800	33	20	16.3—24.3*	upper level 113—167 <sup>i</sup> lowest level 44—61 <sup>ii</sup>
Brynica B I	920	1100	16	14	15.5—26.5** ca 6.5	upper level 78—104 <sup>v</sup> lowest level 28—34 <sup>vv</sup>
Brynica B II	130	133	5	3		
Pszczyna, P (control)	20	40	0.5	1		15—25

Legend: \* except Feb. (5.6), Aug. (9.3) and Nov., Dec. (ca 13)

\*\* except Apr., Oct. (ca 8.5) and Jan., Feb., Dec. (11.9-13.8)

i in Jan, Aug, Now, Dec.

ii in May, June, July, Sep.

v Jan, July, Dec.

vv in May, June, Aug, Oct.

**Methods.** After cutting off the necrotic and injured parts of leaves, the leaves, were ground during 2 min and to every of the four one-gram samples, 25 mililiters of  $\text{H}_2\text{O}$  of pH 6.8-7.0 were added. After extensive mixing, the samples were left in the dark for 24 h and then the measurements of pH and buffer capacity were carried on according to author's description given in the previous work (Czuchajowska, Przybylski, 1978). The pH values were measured with accuracy of 0.05 pH unit. The buffer capacity was expressed as buffer capacity against acid,  $\text{bc}_a$ , and that against base,  $\text{bc}_b$ . The first one equals the number of mililiters of 0.025 n  $\text{H}_2\text{SO}_4$  causing the pH decrease of aqueous leaves homogenates by 1 pH unit, the second one — to the number of mililiters of 0.025 n NaOH necessary to increase pH by the same interval. The correlations pH vs  $\text{bc}_a$  and pH vs  $\text{bc}_b$  were calculated according to Snedecor (1956).

One-year run of research was started at the beginning of November 1976 and ended in November 1977. The samples were collected at the beginning of every month. The measurements were performed during 12 months for the bi- and triennial leaves of *Vaccinium vitis-idaea*, the current year leaves were investigated since their formation in June till the end of research in November. *Vaccinium myrtillus* leaves were investigated in the period of May—October, i.e. until the leaf fall.

## RESULTS

The graphs of seasonal pH changes, Fig. 1, of *Vaccinium vitis-idaea* leaves, beginning with the current year ones, reveal a very characteristic feature: the newly formed leaves show the lowest pH values which gradually tend to increase from the pH range of 3.60—3.75 in July (for all areas) up to 4.30-4.60 in November. The biennial leaves are less acid although they also gradually increase their pH, from 4.40 in November to 4.45-4.70 in November of the next year; they never show the acidity below pH = 4.0. The oldest, i.e. the triennial leaves are characterised by a very limited increase of pH, the lowest one for the Żygliń area: by 0.1 pH unit, the largest one for Brynica II: by 0.3 unit.

In the course of a 6 month long period of research, *Vaccinium myrtillus* leaves show a substantial pH increase from 3.40-3.55 to 4.10-4.60 for all plots investigated; although, at the beginning the leaves appear more acid than the current year ones of *Vaccinium vitis-idaea*, the total pH increase is very similar. The curves of seasonal pH changes for all areas are close to each other, nevertheless — if one consider every area separately — some distinct differences can be noticed, e.g. from the Brynica I plot the leaves of *Vaccinium vitis-idaea* of every age — tend to show the pH values usually lower than the leaves from the remaining polluted plots.

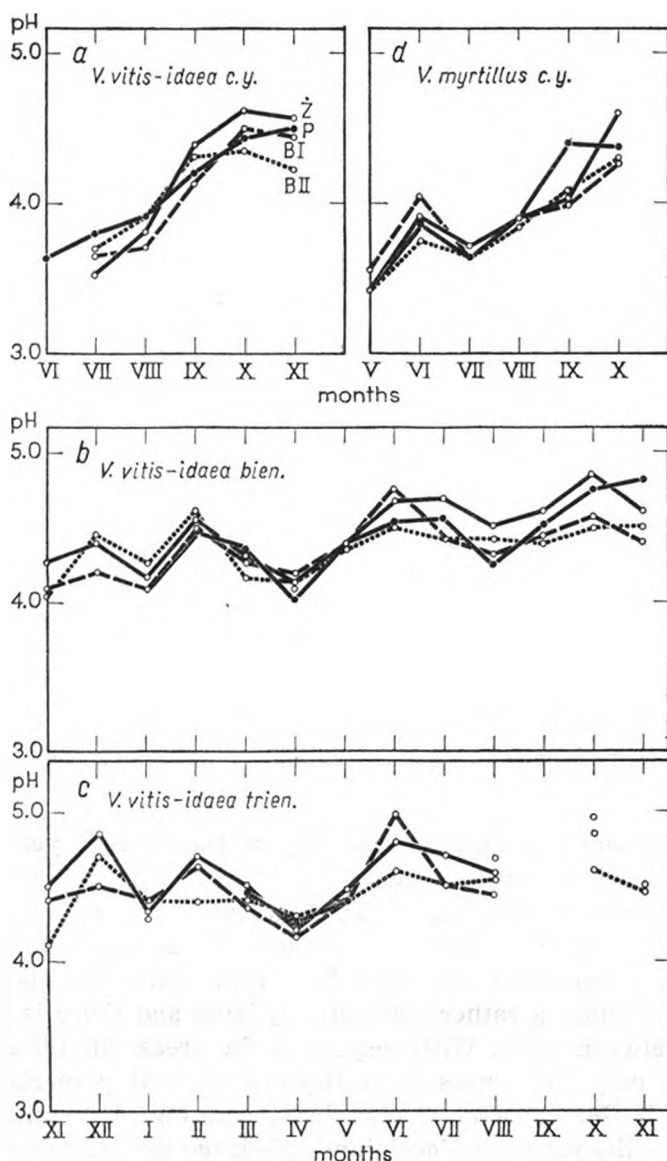


Fig. 1. Seasonal changes of pH of the aqueous homogenates of *Vaccinium* leaves: the current year (a), biennial (b) and triennial (c) leaves of *Vaccinium vitis-idaea*, the current year leaves of *Vaccinium myrtillus* (d) from the stands at Pszczyna, P (control), Żyglinek, Ż, Brynica I, B I, and Brynica II, B II

The difference between the course of the seasonal pH changes in unwashed and washed leaves, all the results discussed above are concerned with the former ones, is not essential, as one can see on the example given in Fig. 2, although the separate values are not identical.

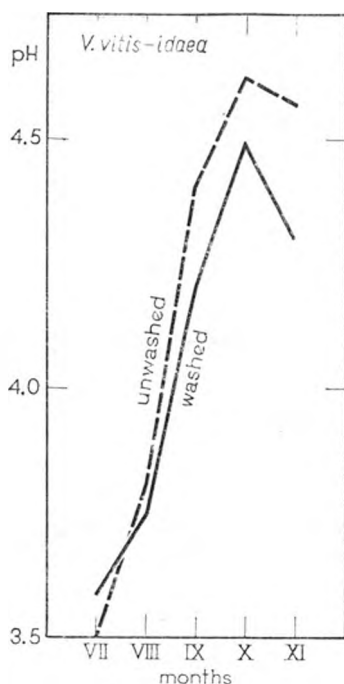


Fig. 2. Seasonal changes of pH of the washed (continuous line) and unwashed (dashed line) current year leaves of *Vaccinium vitis-idaea* from Żygliniek

The buffer capacity against acid,  $bc_a$ , of the current year leaves of *Vaccinium vitis-idaea* shows high level of values in the period June-July, 7.2-8.0 ml/pH unit, Fig. 3. The decrease became more advanced in August, to achieve in November a low  $bc_a$  level, corresponding to that of biennial leaves at this time, 3-4 ml/pH unit. The bi- and triennial leaves maintain a rather constant  $bc_a$  level and there is no distinct difference between them. With respect to the areas, the differences are also limited, only the leaves from Brynica I showing markedly lower values. The buffer capacity against base,  $bc_b$ , Fig. 4, maintains a low level even for the youngest *Vaccinium vitis-idaea* leaves, being two-times or even more lower, particularly in the period of the most intense metabolic processes, i.e. from June till September, than that of the corresponding  $bc_a$  value. The limited  $bc_b$  increase was noticed in the current year leaves in the period of July-September with a maximum in August, 4-4.35 ml/pH unit. In November the  $bc_b$  values of the current

Fig. 3. Seasonal changes of buffer capacity against acid (in ml 0.025 n  $H_2SO_4$  per pH unit), concerning the current year (a), biennial (b), triennial (c) leaves of *Vaccinium vitis-idaea* and the current year ones of *Vaccinium myrtillus* (d); the stands are denoted as in Fig. 1

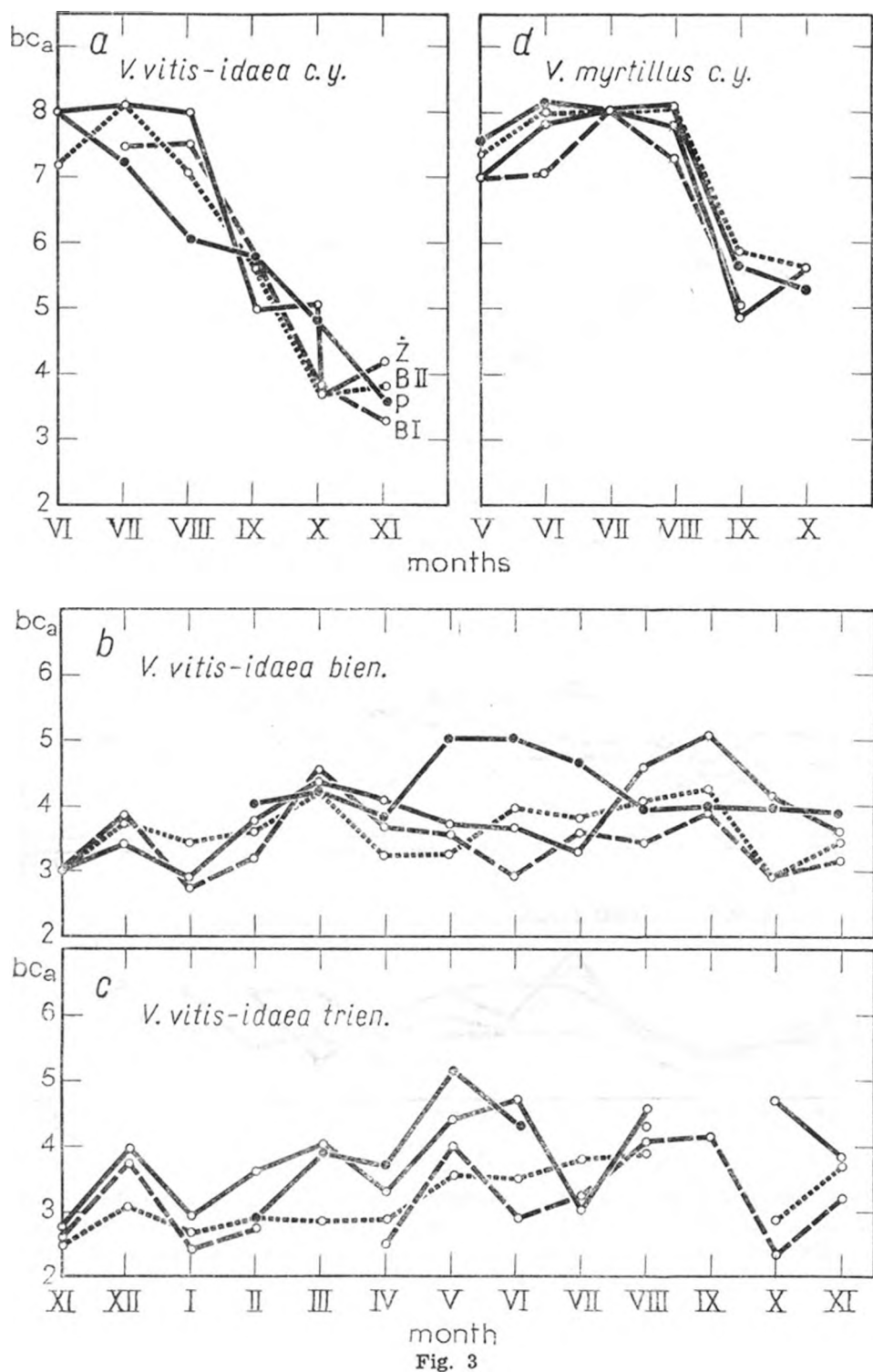


Fig. 3



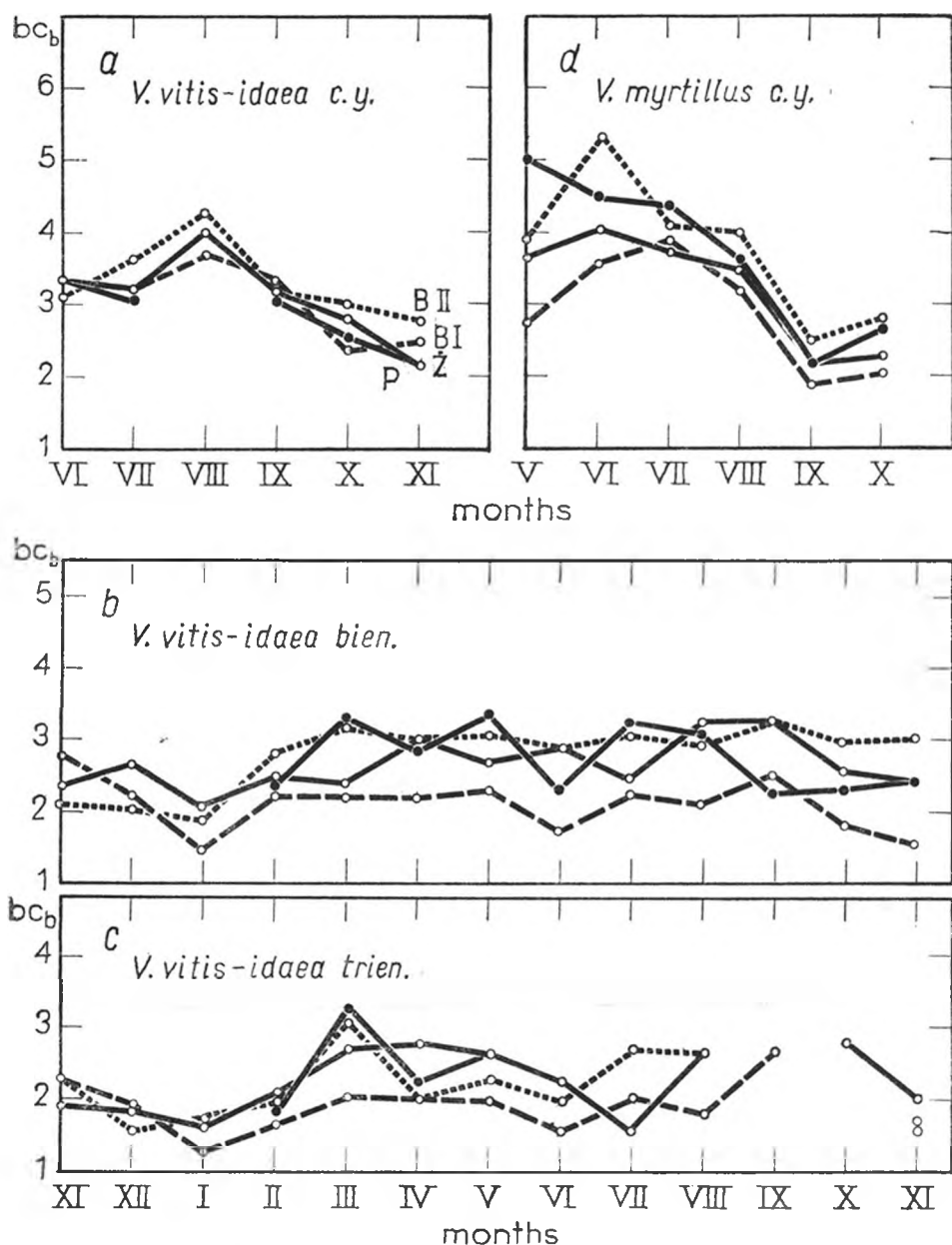


Fig. 4. Seasonal changes of buffer capacity against base (in ml 0.025n NaOH per pH unit), concerning the current year (a), biennial (b), triennial (c) leaves of *Vaccinium vitis-idaea* and the current year ones of *Vaccinium myrtillus* (d); the stands are denoted as in Fig. 1

year leaves equal those of biennial ones and such a low  $bc_b$  level is typical for the triennial leaves.

The course of seasonal  $bc_a$  and  $bc_b$  changes in *Vaccinium myrtillus* leaves is similar to that of the current year ones of *Vaccinium vitis-idaea*, however, during the first four-months (May-August) the former leaves show not only a high  $bc_a$  level, but a constant one, 7-8 ml/pH unit; the decrease began in September. The curves of seasonal  $bc_a$  changes related to different plots form a compact bunch of lines, Fig. 3, however, the  $bc_b$  values are more differentiated, the lowest ones being typical for Brynica I area. Since May till August the  $bc_b$  values of *Vaccinium myrtillus* leaves are 1.5 times smaller than the corresponding  $bc_a$  values.

The calculated correlations between the acidity and  $bc_a$ , and also between the acidity and  $bc_b$ , show very interesting patterns. There exists a dependence between the acidity of the *Vaccinium myrtillus* leaves and the current year ones of *Vaccinium vitis-idaea* and their  $bc_a$  and  $bc_b$  values; for all the areas the correlation coefficients are in the ranges:  $bc_a$  0.80-0.89,  $bc_b$  0.63-0.73. Such correlations were not found for the bi- and triennial leaves of *Vaccinium vitis-idaea*. The correlation of acidity-vs- $bc_b$  which was not noted for the previously investigated species of *Pinus* and *Larix* is worth of noting for the *Vaccinium* species.

## DISCUSSION

The peculiarity of pH changes and particularly of buffer capacity changes in *Vaccinium* species, the leaves of which are susceptible to necrotic changes and chlorosis due to air-pollution (Buszman, Lorek, 1979) is clearly visible when compared with *Pinus*, mainly *Pinus silvestris*, the species co-existing with *Vaccinium* in the investigated ecosystem; a dependence between the changes of pH and buffer capacity of the latter and the intensity of pollution has been already noticed (Czuchajowska, Przybylski, 1978; Czuchajowska, Niemtur, 1978). It occurred that with the age of needles the pH of their homogenates increased, the buffer capacity decreased and a marked correlation of pH vs  $bc_a$  exists. The increase in industrial emissions was reflected in the increase of the buffer capacity of the homogenates. Although for *Vaccinium* and *Pinus* species the range of seasonal pH,  $bc_a$  and  $bc_b$  values is similar, some differences appear, mainly with respect to buffer capacity. The pH changes for *Vaccinium* are less dependent on the type of the stand, i.e. on the intensity of industrial emission than for *Pinus silvestris*, they are also less pronounced for the unpolluted leaves during the whole season, particularly for the current year ones of *Vaccinium vitis-idaea*:  $\Delta$  pH = 0.5 against 1.0 for *Pinus silvestris*.

A remarkable feature is the narrowing of the buffer capacity range of *Vaccinium* leaves from the air-polluted stands; the range found was equal or a little broader than that of the control leaves. The leaves of *Pinus silvestris* behave differently in this respect. For the samples taken from the areas of different degree of air pollution in summer, this species shows the range of  $bc_a$  changes by 150 per cent larger than that of the control leaves, whereas for the *Vaccinium vitis-idaea* and *Vaccinium myrtillus* leaves the increase equals 25 and 30 per cent, respectively. The same trend of changes is visible when the mean  $bc_a$  values of the current year and of the biennial leaves are compared during the summer period: for *Pinus silvestris* the  $bc_a$  value of the young needles is by 270 per cent (taking into account the air-polluted stands) and by 210 per cent (considering only the control stand) higher than that typical for winter, the increases for *Vaccinium vitis-idaea* leaves are 155 and 145 per cent, respectively. Thus, the morphological sign of emission-resistance of *Vaccinium* species being lower than that of *Pinus* corresponds to the markedly lower buffer capacity of the former species and to higher limitations in its ability to increase the buffer capacity under the influence of emissions. Some differences in the extent of the changes occurring under the influence of emissions in a few month-old leaves of *Vaccinium vitis-idaea* and *Vaccinium myrtillus* (in favour of the former ones) can be considered as corresponding to somewhat different behaviour of the two species noticed in phenological research performed on the polluted areas considered in this paper (Buszman, Lorek, 1979). It is also characteristic that the diminishing of the buffer capacity of leaves occurs in winter when the rest period of the plant causes that it does not need an intense protection against changes of acidity. The limited increase of buffer capacity of polluted leaves as compared to that of unpolluted ones would suggest their restricted ability of protection against the pH changes, at any rate more restricted than that shown by *Pinus silvestris*.

The investigations described in the present paper were concerned with aqueous homogenates of *Vaccinium* leaves (in the dark), i.e. with a system in which, at the best, only a part of internal space of chloroplast thylakoids became open and accessible to the measurements. This made impossible to discuss the results in terms presented by Walz et al. (1974) who investigated the internal buffer capacity and proton content in isolated lettuce chloroplasts in the light, finding the dependence on the pH of the solution in which they were suspended, and proving that the buffer capacity changes drastically while the number of protons bound remains rather equal. However, bearing in mind that the present results are the first concerning the *Vaccinium* species of attributed bio-indicative properties with regard to the industrial emissions, it seems that they deserved at least a discussion in general terms. The quan-

titative interpretation of the described phenomena would be only possible as a result of investigations on the pH and buffer capacity changes of chloroplasts isolated from the leaves of *Vaccinium* grown and influenced by pollutions under controlled conditions, being now in progress.

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## Sezonowe zmiany pH i pojemności buforowej wodnych homogenizatów liści *Vaccinium vitis-idaea* i *Vaccinium myrtillus*

### Streszczenie

Liście obydwu badanych gatunków *Vaccinium* pobierane były z trzech stanowisk znajdujących się w odległości od 0.9 do 5 km od huty cynku w Miasteczku Śląskim, różniących się stopniem zanieczyszczeń (Tab. 1) pochodzących od tego emitora. Stanowisko kontrolne znajdowało się w okolicach Pszczyny, odległej o 40 km. Krzywe sezonowych zmian pH wodnych homogenizatów liści dla wszystkich badanych stanowisk wykazują podobny przebieg (Rys. 1), mimo iż pojawiają się wyraźne różnice pomiędzy stanowiskami; liście *Vaccinium myrtillus* oraz młode liście *Vaccinium vitis-idaea* wykazują najniższe wartości pH w czerwcu i lipcu, od 3.4 do 3.7, kwasowość ich maleje z czasem, osiągając w listopadzie pH ok. 4.6. Dwu- i trzyletnie liście *Vaccinium vitis-idaea* są już wyraźnie mniej kwaśne. Różnice sezonowych zmian kwasowości liści mytych i niemytych są raczej niewielkie (Rys. 2).

Pojemność buforowa względem kwasu,  $bc_a$ , wyrażona liczbą mililitrów 0.025 n  $H_2SO_4$  powodujących spadek pH o jednostkę, jest największa dla liści *Vaccinium myrtillus* oraz młodych liści *Vaccinium vitis-idaea* (Rys. 3). Szczególnie wysoka wartość  $bc_a$  pojawia się dla liści *Vaccinium myrtillus* w okresie od czerwca do sierpnia, 7-8 ml/jedn. pH, po tym okresie spada do 3-4 ml/jedn. pH. Liście dwu- i trzyletnie *Vaccinium vitis-idaea* wykazują stosunkowo niewielkie sezonowe zmiany  $bc_a$ . Zmiany pojemności buforowej względem zasady (0.025 n NaOH),  $bc_b$ , kształtują się na ogół podobnie (Rys. 4), niemniej zawsze wartości  $bc_b$  są niższe — czasem nawet dwukrotnie — niż  $bc_a$ , natomiast zróżnicowanie wartości pojemności buforowej w zależności od stopnia zanieczyszczenia stanowiska jest większe dla  $bc_b$  niż  $bc_a$ . Istnieje korelacja pomiędzy kwasowością jednorocznych liści obydwu gatunków a ich pojemnością buforową, odpowiednie współczynniki wynoszą 0.80-0.89 (dla  $bc_a$ ) oraz 0.63-0.73 (dla  $bc_b$ ). Jest nader charakterystyczne, że zmiany  $bc_a$  są znacznie mniej zależne, zwłaszcza w odniesieniu do *Vaccinium myrtillus*, od stopnia zanieczyszczenia stanowiska niż wykazuje to *Pinus silvestris* występujący w tych samych ekosystemach, co odpowiadałoby sugerowanym w literaturze bioindykacyjnym własnościom *Vaccinium*.